Assessment of chemical inhibitor addition to improve the gas production from biowaste

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Abstract

The coexistence of sulphate-reducing bacteria and methanogenic archaea in the reactors during the anaerobic digestion from sulphate-containing waste could favor the accumulation of sulfide on the biogas, and therefore reduce its quality. In this study, the effect of sulphate-reducing bacteria inhibitor (MoO_4^{-2}) addition in a two phase system from sulphate-containing municipal solid waste to improve the quality of the biogas has been investigated. The results showed that although SRB and sulphide production decreased, the use of inhibitor was not effective to improve the anaerobic digestion in a two phase system from sulphate-containing waste, since a significant decrease on biogas and organic matter removal were observed. Before MoO_4^{-2} addition the average values of volatile solid were around 12 g/kg, after 5 days of inhibitor use, those values did exceed to 28 g/kg. Molybdate caused acidification in the reactor and it was according to decrease in the pH values. In relation to microbial consortia, the effect of inhibitor was a decrease in Bacteria (44 %; 60% in sulphate-reducing bacteria) and Archaea (38%) populations.

Keywords: biomethanization; inhibition; sulphate-containing solid waste; microbial community structure.

1. Introduction

Conventional bioconversion of waste in anaerobic digestion (AD) systems is widely recognized (Cuetos et al. 2008; Martín-González et al. 2013; Xing et al. 2014) and is characterized by four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The first three steps are carried out by various bacteria species while the fourth step (methanogenesis) is usually dominated by special microorganisms belonging to the Archaea domain (Zahedi et al. 2016). In the first and second steps, hydrolysis and acidification take place by hydrolytic-acidogenic bacteria (HAB), and intermediate products such as volatile fatty acids (VFA), hydrogen (H_2) and carbon dioxide (CO_2) are generated. In the third step, VFA are transformed into acetate, H_2 and CO_2 by acetogenic bacteria. Usually, Propionate-utilizing acetogens (PUA) and butyrate-utilizing acetogens (BUA) are the majority of the acetogens in the anaerobic reactors (Mara and Horan 2003). The methanogens occupy the terminal position in the anaerobic food chain and are normally divided into two main groups based on their substrate conversion capabilities. Acetoclastic methanogens (AUM) are able to convert acetate into methane and carbon dioxide (Montero et al. 2008). Hydrogenotrophic methanogens (HUM) convert H_2/CO_2 to methane. These species play a key role in the overall process by maintaining the very low partial pressures of H_2 (<10 Pa) necessary for the functioning of the intermediate trophic group, the acetogens, which are responsible for the conversion of acids organic and alcohol intermediates to direct methane precursors (Montero et al. 2008).

During anaerobic treatment of sulphate-containing wastewaters, sulphate-reducing bacteria (SRB) compete for substrate with other anaerobic bacteria or methanogens. Sulphate competes against organic carbon as an electron acceptor and it leads to the undesirable production of hydrogen sulphide (H_2S).

55 H_2S is a corrosive gas and its presence reduces the potency of biogas as a fuel for
56 boilers or electricity generation in a biogas engine. Besides, it greatly affects the
57 flammability of biogas when used directly in burners (Rasi et al. 2007; Muñoz et al.
58 2015). In addition, H_2S causes malodor and health hazards due to the well-known
59 toxicity and material corrosion tendencies (Auguet et al. 2015). The nutritional
60 requirements of SRB include an inorganic electron acceptor which is usually provided
61 by sulphate ion and an electron donor which essentially consist of VFA or H_2 and
62 occasionally sugars and long chain fatty acids.

63 Two stages of inhibition exist as a result of sulphate reduction (Chen et al. 2008): (i)
64 primary inhibition due to competition for common organic and inorganic substrates
65 from SRB and (ii) secondary inhibition which results from the toxicity of sulphide to
66 various microbial groups. In this regard, many researchers have used molybdate (MoO_4^{-2})
67 as sulphide inhibitor where different substrates were utilized (Newport and Nedwell
68 1988; Tucker et al. 1998; Ranade et al. 1999; Isa and Anderson 2005; Predicala et al.
69 2008; Rincon et al. 2008; Biswas et al. 2009; Jesus et al. 2015) and all of them show of
70 molybdate successful in inhibiting SRB activity.

71 Nevertheless, data on the outcome of competition between SRB and the
72 microorganisms mentioned above are contradictory in literature (Chen et al. 2008). The
73 considerable variation in the inhibition/toxicity levels reported for sulphate is due to the
74 complexity of AD process where mechanisms such as antagonisms, synergism,
75 acclimation and competition could significantly affect the phenomenon of inhibition.

76 Studies involving use of inhibitors to suppress activity of SRB and, consequently,
77 promote growth of methanogens have been reported in literature (Ranade et al. 1999;
78 Isa and Anderson 2005; Patidar and Tare 2005; Chen et al. 2008). However, other

studies documented that SRB and methanogens could have a symbiosis between them (Vossoughi et al. 2003; Zahedi et al. 2013a; Auguet et al. 2015), although these organisms are constantly competing as electron acceptors. Therefore, the feasibility of using SRB inhibitor for the control of sulphate reduction and the improvement of methane production in biological reactor are not established.

In view of this, the present study was undertaken to investigate the effect of molybdate supplementation on the two-phase dry-thermophilic AD process of sulphate-containing municipal solid waste. Structure and dynamics of the anaerobic consortia developed along the experiment were analyzed by fluorescent in situ hybridization (FISH) employing different oligonucleotide probes.

2. Methods

2.1 Experimental equipment

Two laboratory-scale continuously stirred tank reactors were employed (Figure 1). The first reactor, dedicated to the hydrogen production (HP) (first phase), had a 5.5 liters working volume, while the second reactor (second phase) dedicated to the methane production (MP) had a 5 liters working volume, both heated by recirculating water through a thermostatic jacket. PRECISTERM 6000142/6000389 (SELECTA S.A.) baths were used, with a maximum capacity of 7 liters of water. The stainless steel reactors lid have a diameter of 200 mm and contain three openings, one for the biogas outlet, a feed inlet and another opening for the stirring system. The bottoms of the reactors had a discharge valve with a 40 mm i.d., used for sampling. The biogas was collected in 40 liter capacity Tedlar (a polyvinyl fluoride plastic polymer) bags. The stirring systems consisted of an IKA EUROSTAR Power Control visc-P4 overhead stirrer coupled to a stainless steel blade with scrapers which allows homogenization of

the waste at a speed of 23 rpm. The system was fed semi-continuously, once per day, and the hydraulic retention times (HRT) were 1.5 (organic loading rate (OLR) = 57 g/l/d) and 5 d (OLR = 8 g/l/d) for the first and second phase, respectively.

2.2 Inoculum, substrate and feeding

The seed used as acidogenic and methanogenic inoculum were collected from a two phase dry-thermophilic system of urban wastes. The total solid (TS) and volatile solid (VS) concentrations in the second phase (methanogenic inoculum) were 67 g/kg and 33 g/kg as against their concentrations in the first phase (acidogenic inoculum) which were 82 g/kg and 50 g/kg, respectively.

The tested substrate in the first phase was the urban wastes from the 30 mm trommel of the municipal solid waste treatment plant in Cadiz, Spain. The urban wastes was stored in 25 kg drums at - 4º C to avoid AD by the microorganisms found in the solid waste itself (Zahedi et al. 2013b). The TS concentration of the feed of the first reactor was adjusted to 20 % (which is characteristic of dry AD) by adding tap water. Composition of the substrate (urban solid wastes and water; 20 % of TS) employed in the first phase is shown in Table 1. The substrate used in the second phase was the effluent of the first phase (Table 2). Both reactors were fed once a day (semi-continuous).

2.3 Inhibitor treatment methodology

The effect of continuous dosing molybdate (MoO_4^{-2}) (2.5 mM) to improve the performance in two-phase dry-thermophilic AD of sulphate-containing urban waste was realized. No molybdate was added to the first phase, because under acid conditions the biogas was sulphide-free (Zahedi et al. 2013b). The whole experiment length was 50 d. During the first 45 d no inhibitor was used. In these 45 days two different period were considered: startup (0-20 d) and control/stationary phase (20-45 d). On day 46, sodium

127 molybdate (MW=205.92 g/mol), 2.6 g, was added to the digester so as to have 2.5 mM
128 concentration of the inhibitor in the reactor (V= 5 1) (Isa and Anderson 2005). From
129 next day onwards, i.e. from day 47, based on the daily wash out, 0.52 g per 1000 ml of
130 daily feed was added every day, so as to maintain the 2.5 mM inhibitor concentration in
131 the digester.

132 **2.4 Analytical methods**

133 Total and soluble chemical oxygen demand (TCOD, SCOD), alkalinity, sulphate, VS,
134 pH and VFA were performed according to previous studies (Zahedi et al. 2013c;
135 Dahunsi et al. 2016a,b). Determination of total and partial alkalinity and ammonia
136 (NH_4^+–N) were carried out daily. Fluctuation in the concentration of volatile fatty acids
137 (VFA) was determined using a gas chromatography (GC2010) to which was attached a
138 Fused Silica Capillary Column (Supelco NUKOLTM, 15 x 0.53 x 0.5 µm film
139 thickness) and with a flame ionization detector (200^o C) with H_2 as the carrier gas. An
140 initial temperature of 80^o C was used and was subsequently increased to 140^o C, then
141 160^o C and finally to 200^o C at a rate of 10^o C/min. The analyzed samples were
142 centrifuged and filtered through a 0.45 µm membrane.

143 Production of gas was continuously measured using a gas flow meter (Ritter Company,
144 drum-type wet-test volumetric gas meters), and the composition of the produced gas
145 was determined by gas chromatography separation (SHIMADZU GC-2010). H_2, CH_4,
146 CO_2, O_2 and N_2 were analyzed by means of a thermal conductivity detector (TCD)
147 employing a Supelco Carboxen 1010 Plot column. Samples were taken using a 1 ml
148 Dynatech Gastight gas syringe under the following operating conditions: split = 100;
149 constant pressure in the injection port (70 kPa); 2 min at 40 ºC; ramped at 40 ºC/min
150 until 200º C; 1.5 min at 200º C; detector temperature: 250º C; and injector temperature:

200º C. Helium was used as carrier gas (266.2 ml/min) (Zahedi et al. 2017b) . Commercial mixtures of H_2, CH_4, CO_2, O_2, N_2 and H_2S (Abelló Linde S.A.) were used to calibrate the system.

2.5 Microbiological analysis and biochemical activity

The cellular concentration and percentages of Bacteria and Archaea were quantified by epifluorescence method (FISH) according to the method of Zahedi et al. (Zahedi et al. 2013c; Zahedi et al. 2017a). The main steps of FISH of whole cells using 16S rRNA-targeted oligonucleotide probes are cell fixation, permeabilisation and hybridisation with the desired probe(s).

The cellular concentration and percentages of *Eubacteria*, *Archaea*, BUA, PUA, SRB, HUM and AUM were obtained by FISH according to Zahedi et al.(Zahedi et al. 2013c; Zahedi et al. 2014). The total population was calculated as the sum of the relative amounts of *Eubacteria* and *Archaea*, because the main anaerobic groups in the anaerobic reactors are contained within these two domains (Griffin et al. 1998). Acetogens were calculated as the sum of the relative amounts of PUA and BUA. HAB were calculated as the difference in the relative amounts of *Eubacteria* and acetogens.

3. Results and discussion

The process performances and the functional *Bacteria* and *Archaea* community structures of the two-phase anaerobic reactors for HP and MP were investigated and analyzed together. The section has been structured into two parts: hydrogenic phase performance and methanogenic phase performance.

3.1 Hydrogenic phase performance

As commented before, no molybdate was added to the first phase, because the biogas was sulphide-free. The characterization physical-chemical and microbiological in the

176 effluent of the first phase are shown in the Table 2. The performance in the first phase
177 was according to Zahedi et al.(Zahedi et al. 2013c) study to 1.5 d HRT. This pH value
178 was close to the ideal pH conditions for HP of 5.5 (Bolzonella et al. 2012). VFA
179 composition was composed mainly of butyric acid (5.1±0. g/l). The dominant
180 fermentation products were butyric acid and acetic acid with small amounts of propionic
181 also detected (<0.1 g/l). The sulphate values in the effluent were the same with those
182 measured in the feeding; therefore no sulphate consumption was detected (no SBR
183 activity was detected). A high solubilization (increase in SCOD and VFA and decrease
184 in VS) was detected. The butyrate acid concentration was higher than acetic acid
185 concentration and it was in line to other researches of hydrogen production from similar
186 wastes (Cadiz-Spain urban wastes) (Romero Aguilar et al. 2013; Zahedi et al. 2013b;
187 Angeriz-Campoy et al. 2017; Zahedi et al. 2017a). The ratio of butyrate:acetate (g
188 butyrate/g acetate) was 2.5 and it according to the ratios reported by these previous
189 studies. The biogas produced was composed of H_2 and CO_2 without CH_4 and H_2S
190 detection. In terms of yields, biohydrogen in the first reactor was 47 ± 4% and the HP
191 was 2.0 ± 0.3 L H_2/l/d).

192 3.2 Methanogenic phase performance

193 *3.2.1 Process stability*

194 The stability of the process was evaluated based on the evolution of pH and the
195 VFA/alkalinity ratio (VFA/Alk) before (1-45 d) and after (45-50 d) SRB inhibitor
196 addition (Siles Lopez et al. 2009).

197 Fig. 2.a shows the evolution of pH throughout the test. At the beginning (before
198 inhibitor addition) the systems were able to self-regulate and reach a pH of between 7.0
199 and 8.5, the optimal pH for the activity of methanogens (De La Rubia et al. 2009;

200 Dahunsi et al. 2016). After inhibitor addition, the pH of the reactor decreased below 7.0
201 showing microbial inhibition of H_2 and acid consumer organisms.

202 Alkalinity is the capacity to neutralise acids, the total volatile fatty acids (TVFA)/Alk
203 ratio being typically used as a measurement to evaluate anaerobic system stability
204 (Balaguer et al. 1992; Rincón et al. 2008; Siles Lopez et al. 2009). Values between 0.1
205 and 0.4 (equiv. acetic acid/equiv. $CaCO_3$) indicate favourable operating conditions
206 without the risk of acidification. The evolution of these ratios is shown in Fig 2.b.
207 Before inhibitor addition stability was observed, however from the day after the
208 molybdate was added this ratio decline sharply, demonstrating the non-stability of the
209 second phase (ratios were higher than 0.4). Therefore, the pH values and the acids
210 gathered, thus preventing the activity of the methanogens, as will be explained later.

211 *3.2.2 Process performances*

212 The highest values for the removal of VS (81±7 %) and SCOD (58±2 %) were obtained
213 before inhibitor addition. These values were similar to those obtained by Brownie
214 (Browne et al. 2014) in biomethane production from the organic fraction of municipal
215 solids waste in semi-continuous systems. Before MoO_4^{-2} addition the average values of
216 SCOD and VS in the effluent were around 9 g/l and 12 g/kg respectively, after MoO_4^{-2}
217 inhibition on day 50 those values did exceed to 25 g/l and 28 g/kg respectively (Figure
218 3). The reduction in the consumption of the organic matter means inhibition of the
219 anaerobic digestion process.

220 In relation to VFA, the effect of molybdate was an acidification in the reactor (increase
221 in acid content, Figure 4) according to decrease in the pH values. In the present
222 research, before molybdate addition, the concentrations of VFA were in the order
223 propionic >acetic > butyric. Addition of molybdate caused butyric and acetic acids to

dominate over propionic acid at the end of the trial. The presence of acetic acid in the effluent shows that non-availability of AUM substrate was not the underlying problem for the inhibition of CH_4 production in these studies, but chronic inhibition of AUM by MoO_4^{-2}. In addition, butyric increment in the effluent was resulted of the non-availability to the BUA to consume the butyric. The amount of propionic acid generated after molybdate addition was similar to the amount produced before of inhibitor addition, suggesting that acetogenic activity of PUA was sufficient to achieve the normal propionic acid concentrations(Zahedi et al. 2013a).

It can clearly be observed that the addition of sodium molybdate on day 45 caused immediate inhibition of sulphate reduction, thereby resulting in increase of sulphate content in the effluent (Figure 5) and total absence of H_2S in the biogas (Figure 6a) on the following day. Before MoO_4^{-2} addition the average values of sulphate were around 0.7 g/l and on day 50 those values increased to 2.0 g/l. The values of H_2S decreased from 45±5 mL H_2S/l/d on day 45 to 0 mL H_2S/l/d on day 46. Regarding to the MP, the values of the MP also involved a decrease due to inhibitor use (Figure 6b). Before the use of the SRB inhibitor, MP was around 3.5 L CH_4/l/d and after the supplement it was modestly decreased, until the end, where MP did not exceed 0.2 L CH_4/l/d (day 50). It technically shows that adding this amount of inhibitor for this amount of time does not improve biogas production. Future efforts could incorporate dosing periods with smaller amounts of inhibitor added in increasing increments and the observed return of the system to the previous rate of biogas production and quality.

3.2.3 Microbial community

The evolution of the main microbial group involved in the methanogenic process is described in the Table 3. In the present research, concentrations of different microbial

groups were evaluated before and after inhibitor addition. All the results shown are average values. Before inhibitor addition, the ratio of *Eubacteria: Archaea* was 55:45. These results are in accordance to those obtained by Zahedi et al.(Zahedi et al. 2013c) in the second phase reactor of dry-thermophilic anaerobic digestion process of sulphate-containing municipal solid waste and logically, lower than those obtained by Griffin et al.(Griffin et al. 1998) and McMahon et al.(McMahon et al. 2001) in single-phase reactors of organic waste. SRB population values were in line with those (20-28%) obtained by Zahedi et al.(Zahedi et al. 2013c) and Zhang et al.(Zhang et al. 2011) lower than those (14%) obtained by Mohan et al.(Mohan et al. 2005).

It should be noted, that after inhibitor addition the microbial proportion in the reactor had not very altered, the microbial consortia and microbial activity were hardly altered. It is necessary to emphasize that although the proportion of microorganisms in the reactor is a key, not only the stability, but also the adequate dynamics (‘‘flexibility’’) of the microbial community structure and high values of microbial activity are important for the stable performance of the reactors treating urban wastes[13].

At the end of the trial, the microbial consortia were decreased in 42%. The removal rates of *Bacteria*, *Archaea*, HAB, acetogens, AUM, HUM, SRB, BUA and PUA were 44 %, 38%, 48%, 39%, 35%, 41%, 60%, 63% and 15% respectively. The most affected were SRB and BUA and the most resistant group was PUA which is in line with the constant values of propionic acid in the effluent. The reductions in the microbial consortia, the decrease in the MP, H_2S production, pH value, and increase in the sulphate, VS, SCOD and VFA contents all reveal a decrease in the microbial activity, except for PUA.

In short, the use of the toxic to improve the biogas of the two-phase dry-thermophilic anaerobic digestion process of sulphate-containing municipal solid is not effective. However, it is to be noted that the harmful effect of molybdate supplementation on the SRB and methanogens and therefore, on the H_2S and CH_4 generation makes this treatment an interesting option on other fields, as sewerage system. Since, anaerobic conditions in sewer pipes favor the accumulation of both H_2S and CH_4 and these compounds have detrimental effects on the sewer system, with different consequences for both the installation and its surroundings (Auguet et al. 2015) (such as malodor, health hazards due to the well-known toxicity of H_2S, and corrosion of both the inner surface of pipes and the inlet zones of waste water treatment plants, etc)

Conclusion

Inhibitor addition has proven successful to remove the undesirable production of hydrogen sulphide (H_2S). However it too resulted in an increase in TVFA/Alk ratio, as well as decrease in pH, organic removal organic matter and biogas generation. All this indicated inhibition of all the steps to AD (except to propionic degradation). Therefore, although molybdate is an effective bactericide for SRB, the use of the toxic would be avoided, since molybdate supplementation did not improve the quality of the biogas, under the circumstances of this experiment. Microbial consortia were decreased in 42%. The removal rates of *Bacteria*, *Archaea*, HAB, acetogens, AUM, HUM, SRB, BUA and PUA were 44 %, 38%, 48%, 39%, 35%, 41%, 60%, 63% and 15% respectively.

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Table 1. Physical-chemical and microbiological characterization of the substrate employed in the first phase.

Parameter	Value
pH	5.3 (0.6)
TS (g/l)	120 (15)
VS (g/l)	85 (7)
Sulphate (g/l)	1.9 (0.3)
VFA (g acetic acid/l)	1.8 (0.5)
Acetate (g/l)	1.87(0.5)
Propionate (g/l)	0.0 (0.0)
Butyrate (g/l)	0.4 (0.2)
Total population (10^8 cells/ml)	6 (2)
Eubacteria (%)	78 (3)
Archaea (%)	22 (2)

Average values are shown, with standard deviations in parentheses.

446 Table 2: Physicochemical and microbiological characterization of the first phase reactor
447 effluent

Physicochemical parameters

pH	SCOD (g/l)	VS (g/kg)	Alkalinity ($gCaCO_3$/l)	Sulphate (g/l)	TVFA (g acetic/l)	Acetic (g/l)	Propionic (g/l)	Butyric (g/l)
5.3±0.3	33±2	42±5	4±0	1.9±0.2	10.4±0.9	2.5±0.4	0.1±0.1	5.1±0.5

Microbiological parameters

Total population (10^8 cells/mL)	*Eubacteria* (%)	HAB (%)	Acetogens (%)	BUA (%)	PUA (%)	SRB[a] (%)	*Archaea* (%)	AUM (%)
9.5±0.6	88±2	70±2	18±2	8±1	10±1	14±1	12±1	5±0

448 [a]Percentages compared to total *Eubacteria*.

449

450

451 Table 3: Microbiological characterization of the second phase reactor effluent.

Parameter	Period (Day)					
	1-45*	**46**	**47**	**48**	**49**	**50**
Microbiological parameters						
Total population (10^8 cells/mL)	21.8±2.5	16.3	14.5	14.9	13.9	12.8
***Eubacteria* (%)**	55±2	56	63	51	49	53
HAB (%)	28±1	34	37	27	25	25
Acetogens (%)	27±2	22	26	24	24	28
BUA	14±1	6	9	8	7	9
PUA (%)	13±1	16	17	16	17	19
SRB[a] (%)	26±2	18	18	16	17	18
***Archaea* (%)**	45±2	44	37	49	51	48
AUM (%)	24±1	24	21	29	28	27
HUM (%)	21±1	20	15	21	23	21

452 [a]Percentages compared to total *Eubacteria*.

453 *Values corresponding to the analytical determinations in steady conditions (between day 21 and 45).

454

455 **Figure Captions**

456 Figure 1: The laboratory-scale reactors used in this study. Hydrogenic reactor to the left
457 and methanogenic reactor to the right.

458 Figure 2: (a) pH evolution. (b) TVFA/Alk evolution (g acetic/g $CaCO_3$)

459 Figure 3: (a) SCOD evolution (g/l). (b) Volatile Solid evolution (g/kg).

460 Figure 4: (a) VFA evolution (g/l).

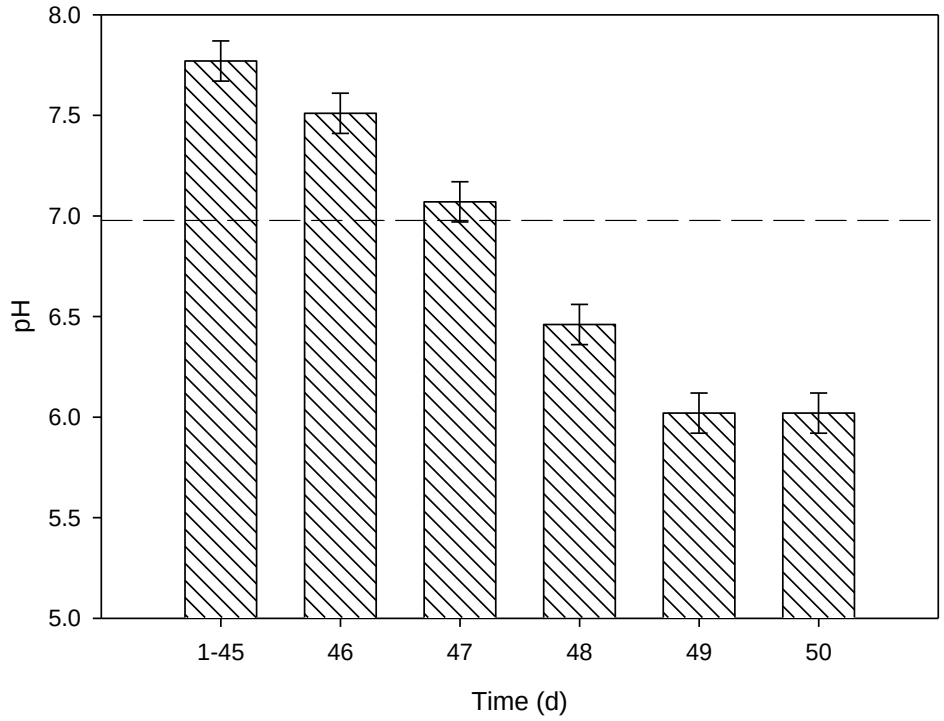
461 Figure 5: (a) Sulphate evolution (g/l).

462 Figure 6: (a) Sulphide production (SP) evolution (ml H_2S/l/d). (b) Methane production
463 (MP) evolution (l CH_4/l/d).

464

Figure 1.

468 Figure 2a



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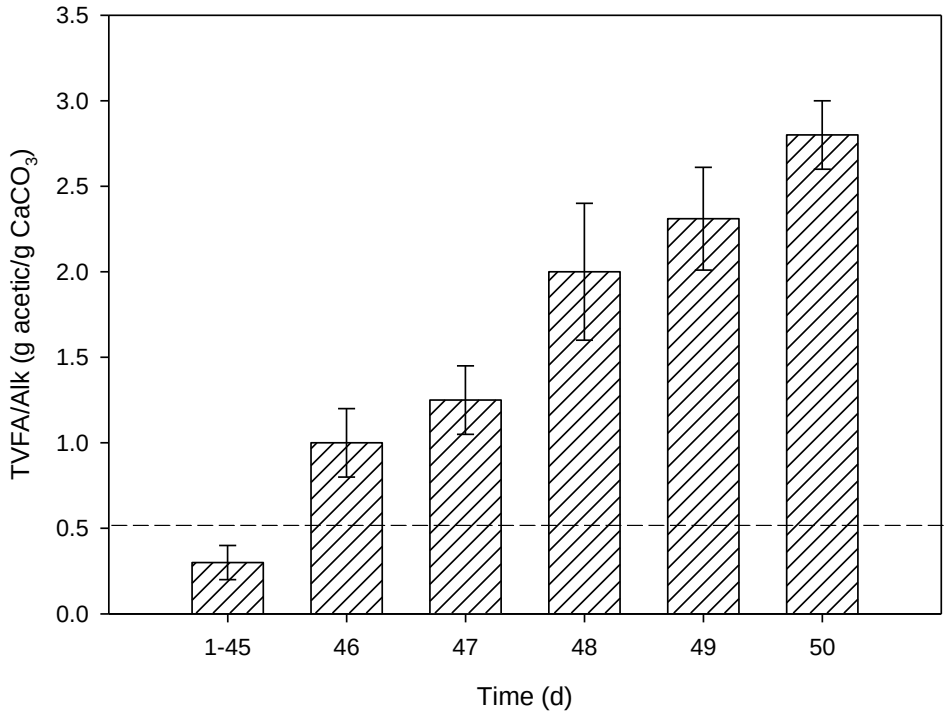
470 Figure 2.b



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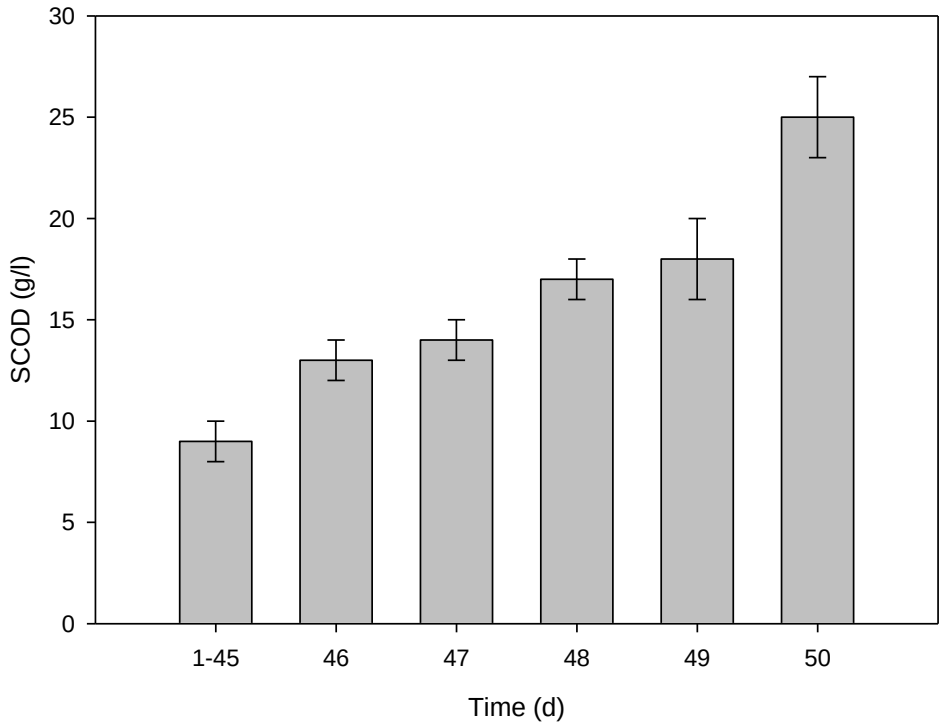
472 Figure 3.a



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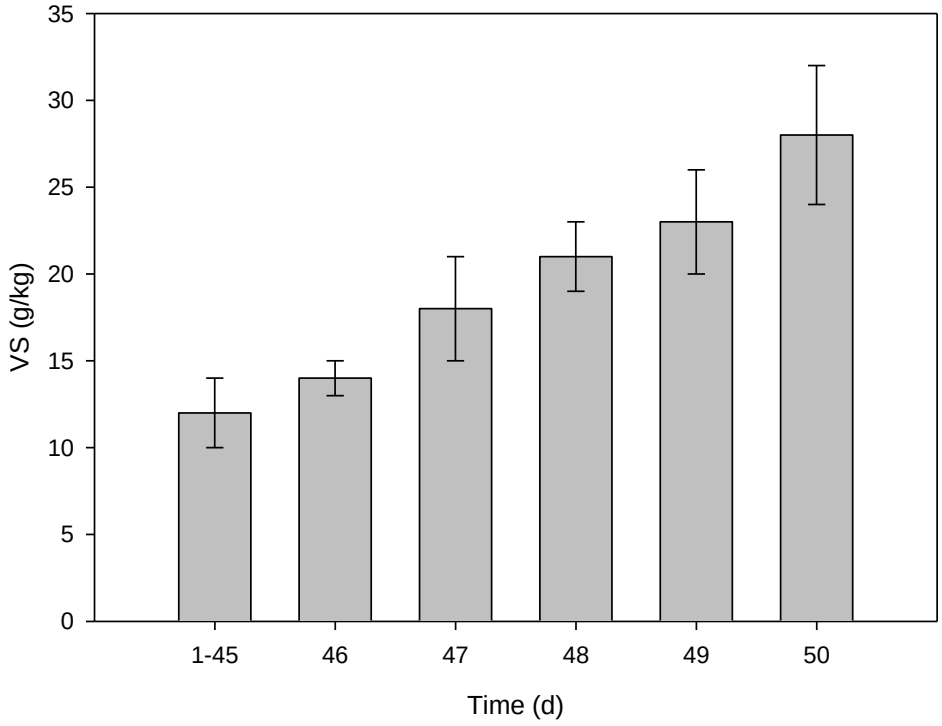
474 Figure 3.b



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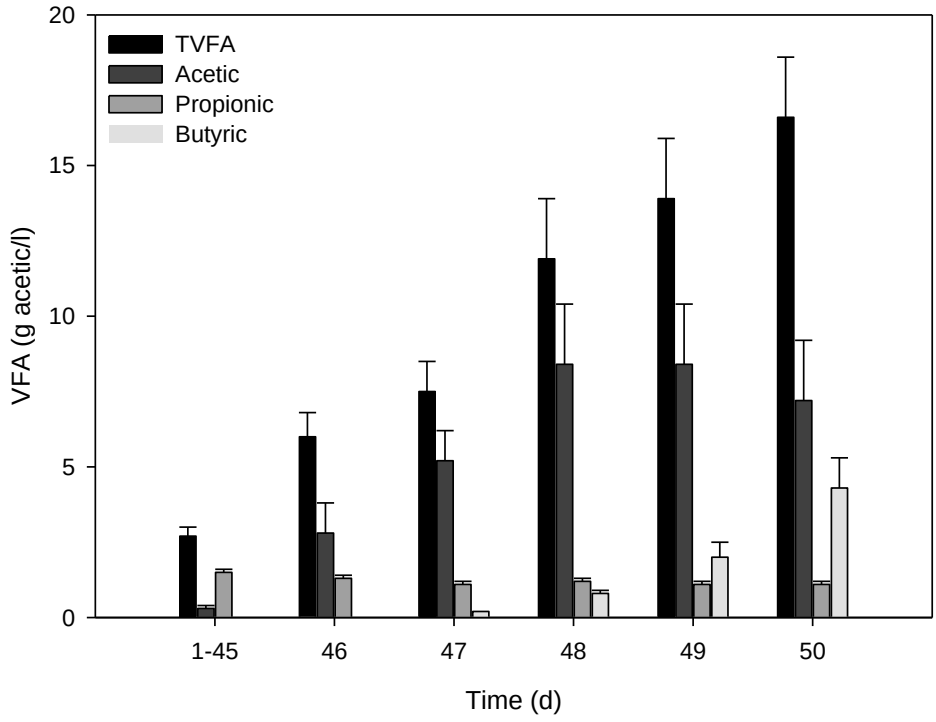
476 Figure 4



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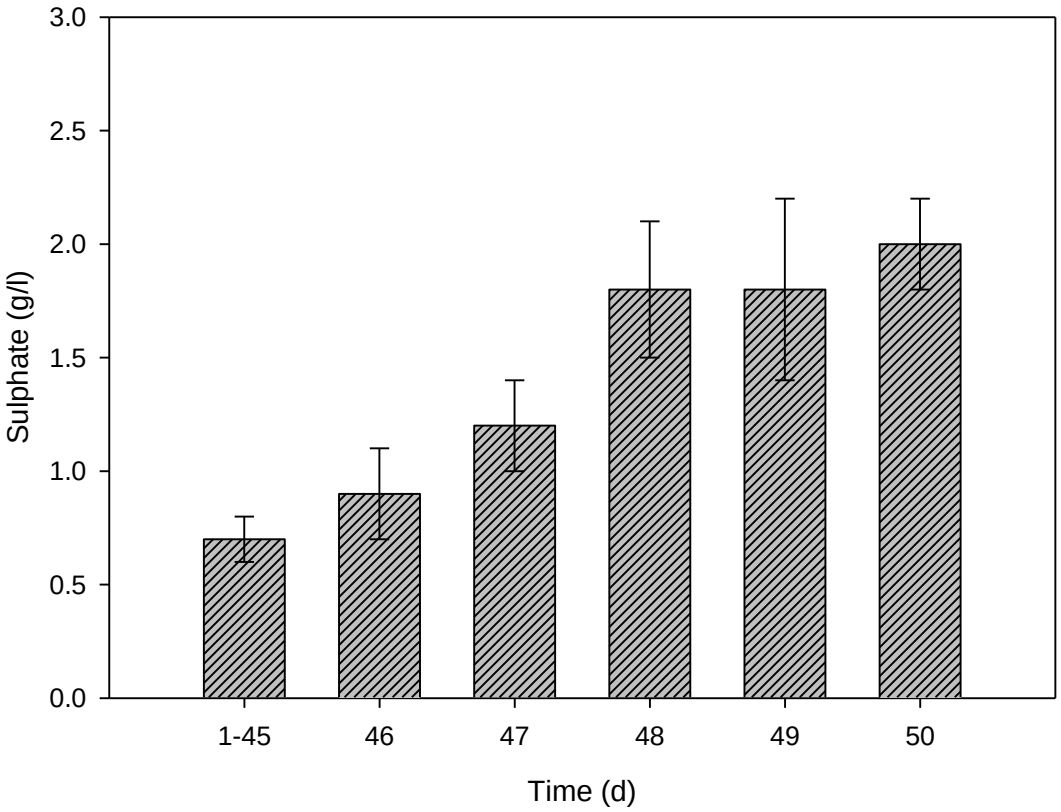
478 Figure 5



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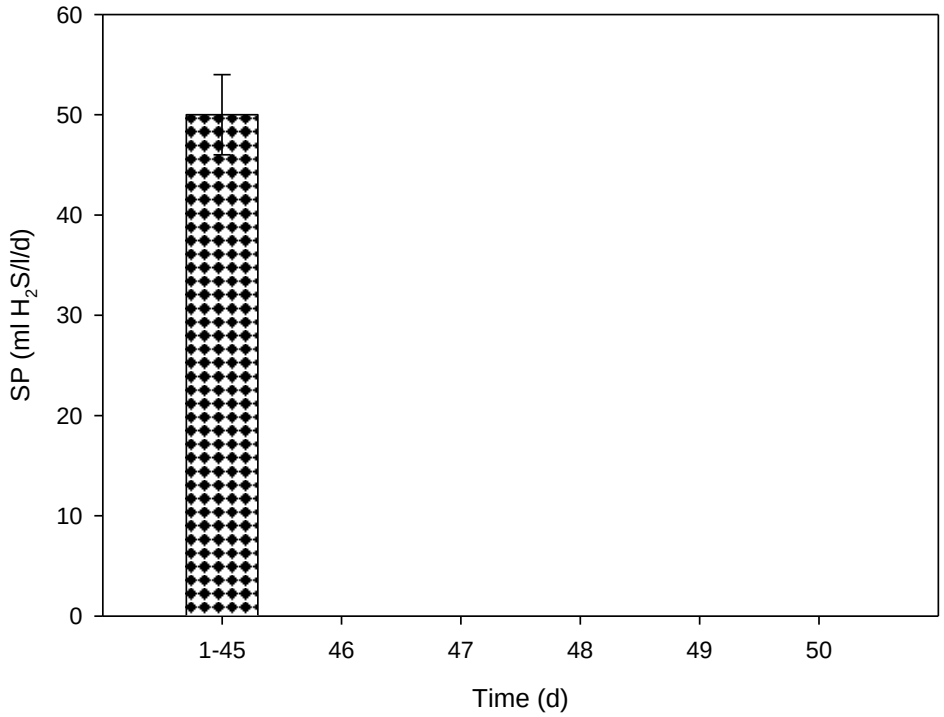
482 Figure 6.a



483
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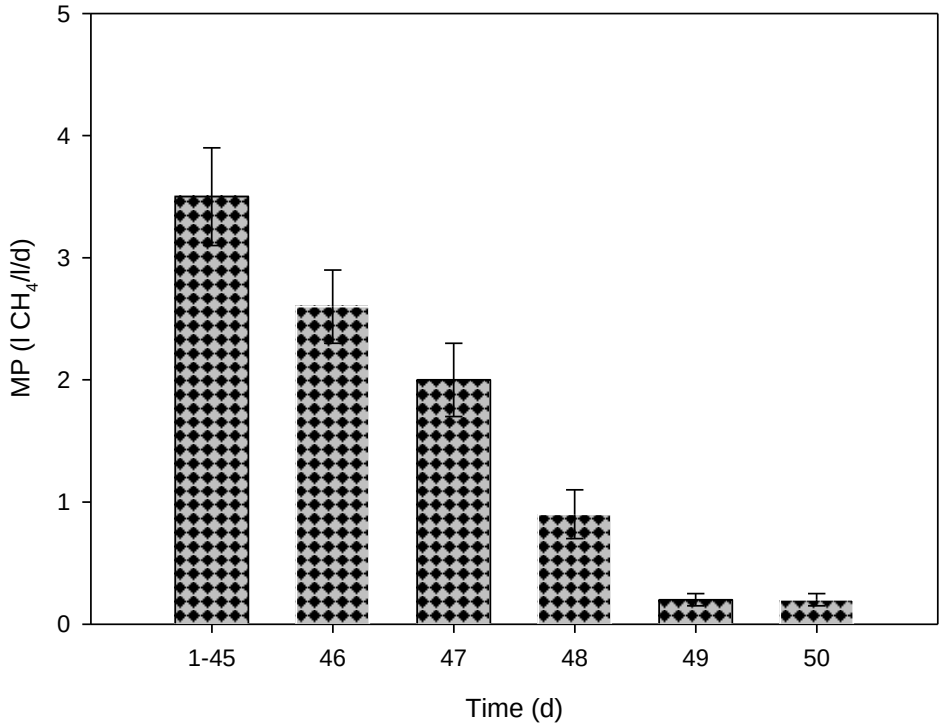
485 Figure 6.b



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